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APPLICATION NUMBER: *60/503,881*
FILING DATE: *September 22, 2003*

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)

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☐ Additional inventors are being named on the ____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

ORAL COMPOSITIONS AND ROUTE OF ADMINISTRATION FOR THE DELIVERY OF A THYLAKOID EXTRACT

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification Number of Pages 6
☒ Drawing(s) Number of Sheets 2
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
☐ Other (specify)

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)

☒ Applicant claims small entity status. See 37 CFR 1.27.
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.
☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE Julie Gauvreau

Date 09/19/03

TYPED or PRINTED NAME JULIE GAUVREAU, PATENT AGENT

REGISTRATION NO. (if appropriate) 52,532

Docket Number: 12893.11

TELEPHONE 514-397-4374

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C.

TITLE OF THE INVENTION

Oral compositions and route of administration for the delivery of a thylakoid extract

FIELD OF THE INVENTION

This invention relates to oral administration of a thylakoid extract or of composition comprising same.

BACKGROUND OF THE INVENTION

Thylakoids are specialized membranes that are responsible for photosynthesis in eukaryotes (plant & algae) and prokaryotes cells (bacteria). These photosynthetic organisms convert CO_2 to organic material by reducing this gas to carbohydrates in a complex set of reactions. Electrons for this reduction reaction utimately come from water, which is then converted to oxygen and protons. Energy for this process is provided by light, which is absorbed by pigments (primarily chlorophylls and carotenoids).

The initial electron transfer (charge separation) reaction in the photosynthetic reaction center sets into motion a long series of redox (reduction-oxidation) reactions, passing the electron along a chain of cofactors and filling up the "electron hole" on the chlorophyll, much like in a bucket brigade. All photosynthetic organisms that produce oxygen have two types of reaction centers, named photosystem I & photosystem II (PSI and PSII) both of which are pigment/protein complexes that are located in thylakoids membrane.

Recently a dynamic and intact thylakoid membrane extract having both anti-oxidative and anti-inflammatory properties and its use in combination with other anti-inflammatory compounds have been described in International patent publication numbers WO 01/49305 and WO 03/04042 , respectively. The anti-oxidative and anti-inflammatory properties of the thylakoid extract have been demonstrated in *in vitro, ex vivo, in situ* and *in vivo* studies. Specifically, the thylakoid extract has been shown to capture the noxious reactive oxygen species including singlet oxygen species and to modulate pro- and anti-inflammatory cytokines toward attenuation of inflammation.

In vivo, topical applications (direct application at site of injury) of the thylakoid extract have been shown to prevent or reduce the UV-induced skin damages in hairless mice and to decrease TPA-induced ear inflammation in rats and mice as

well as preventing damage to intestinal mucosa induced by TNBS or DSS in rats. Also, intraperitoneal injection of the thylakoid extract has been shown to reduce carrageenan-induced paw oedema. However, today, no data has confirmed the potential use of the thylakoid extract as an oral anti-oxidative and/or anti-inflammatory agent.

The present invention relates to the use of a thylakoid extract as an oral therapeutic agent.

SUMMARY OF THE INVENTION

The present invention provides a new use for a thylakoid extract, that is for oral route of administration, and a composition comprising the thylakoid extract in adjunction with an acceptable carrier for oral administration. Besides the pharmaceutical use, the thylakoid extract enters the composition of food or food supplements, for its inocuity and its capacity to provide a diet enriched in anti-oxidants and anti-inflammatory compounds.

Therefore, in accordance with the present invention is provided the use of a thylakoid extract in the making of an oral composition for treating or preventing a disease or disorder involving the formation of reactive oxygen species or inflammation. Also is provided a method for treating or preventing a disease or disorder involving the formation of reactive oxygen species or inflammation in an individual, which comprises the step of orally administering an effective dose of a thylakoid extract. Further is provided an oral composition comprising a thylakoid extract and a vehicle for oral ingestion or oral administration.

DESCRIPTION OF THE INVENTION

Demonstration will be made hereinbelow that the thylakoid extract is active when orally administered.

METHODOLOGY

Animals

Male Wistar rats (180-200g) were used in the experiments. The animals were purchased from Charles River Canada (St-Constant, Qc, Canada). The animals were housed in an environmentally (t = 25°C) and air humidity (60%) controlled room with a 12 h light-dark cycle, kept on a standard laboratory diet and drinking water ad libitum. The experiments were approved by the ethical comittee of TransBIOTech (Levis, Qc, Canada).

Reag nts

12-O tetradecanoyl phorbol 13-acetate (TPA, P-8139) and carrageenan (C-1138) were purchased from Sigma Chemical Co. (St-Louis, MO, USA).

Preparation of the thylakoid extract

The thylakoid extract was obtained from spinach leaves (*Spinacia oleacea*) as described in International patent publication WO 01/49305, the whole content of which is incorporated herein by reference. The thylakoids integrity was evaluated by spectrophotometry (Beckman DU 640) (**Lichtenthale 1987**) and fluorimetry (Hansatech Instruments Ltd, England) (**Maxwell 2000**).

Protocol 1: TPA-induced rat ear oedema

Male Wistar rats (180-200g, Charles River) were fasted overnight (18h). Oedema was induced in the right ear of rats by topical application of 6 ug/ear of TPA in acetone (**Yamamoto S et al. 1994**). The left ear (control) received vehicle (acetone, 20 ul).

Six hours after TPA application, rats were anesthetized (pentobarbital; 80 mg/kg) and a 6 mm diameter disc from each ear was removed with metal punch. The swelling induced by TPA was assessed as the increase in thickness (in mm) of the right ear punch biopsy over that of the left ear and called the oedema index.

The thylakoid extract (25 mg/kg) was administered directly to the duodenum (5ml/kg) via a catheter previously inserted into the duodenum. Physiology saline was administered for control groups (5ml/kg).

Protocol 2: Carrageenan-induced rat paw oedema

Male Wistar rats (180-200g) which had been fasted overnight (18h) received the thylakoid extract (25 mg/kg in sterile physiologic saline) by gavage (5ml/kg) immediatly prior to subplantar injection in the right hind paw of carrageenan (100 ul of 1% supension in 0.9% saline) (Boughton-Smith et al. 1993),or by catheter for an *in situ* release as in protocol 1.

Paw circonference was measured immediately prior to carrageenan injection and also 5 h afterwards. Oedema was expressed as the increased in paw circonference (in mm) measured after carrageenan injection and compared to the pre-injection value for individual animals.

Statistical analysis

Data are presented as mean ± standard error of the means. Mean differences between groups were compared by t-test (SigmPlot 2001 for Windows Version 7.101).

RESULTS

Effect of thylakoids on TPA-induced ear oedema in rats.

Topical application of TPA in control rats induced an increase in ear thickness (50%) over 6 h (figure 1). Simultaneous administration of thylakoids (25 mg/kg given directly into the duodenum via an inserted catheter) reduced (45%) significantly ear oedema induced by TPA.

Effect of thylakoids on carrageenan-induced rat paw oedema

The subplantar injection of carrageenan in control rats induced an increase in paw circunference (5.63 ± 1.29) over 5 h (figure 2). Simultaneous treatment with the thylakoid extract (25 mg/kg) directly into duodenum via a previously inserted catether or by gavage (5 ml/kg), inhibited oedema by 54% and 65%, respectively.

The above results show that the thylakoid extract can be administered enterally or orally. In inflammation models like TPA-induced rat ear oedema and carrageenan-induced rat paw oedema, a decrease of oedema of about 50 % was observed at a dose of 25 mg/kg. Thus it is presumed that a dose of 10 to 10000 mg p.o. per day of thylakoids could be used alone or in combination with any other adjuncted pharmaceutical compound. The intended use is pharmaceutical as well as in food industry as food supplement, additive, preservative or as nutrient *per se*.

The invention being hereinabove described, it will be obvious that the same be varied in many ways. Those skilled in the art recognize that other and further changes and modifications may be made thereto without departing from the spirit of the invention, and it is intended that all such changes and modifications fall within the scope of the invention, as defined in the appended claims.

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Lichtenthaler H.K. (1987), Chlorophylls and carotenoids : Pigments of Photosynthetic Biomembranes In : Packer L. and Douce R. (eds.) Methods in Enzymology, vol 148 pp 350-382. Academic Press, London.

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Claims

1. The use of a thylakoid extract in the making of an oral composition for treating or preventing a disease or disorder involving the formation of reactive oxygen species or inflammation.

2. A method for treating or preventing a disease or disorder involving the formation of reactive oxygen species or inflammation, in an individual, which comprises the step of orally administering an effective dose of a thylakoid extract.

3. An oral composition comprising a thylakoid extract and a vehicle for oral ingestion or oral administration.

Effect of oral administration (via duod num) of thylakoids on TPA-induc d ar o dema

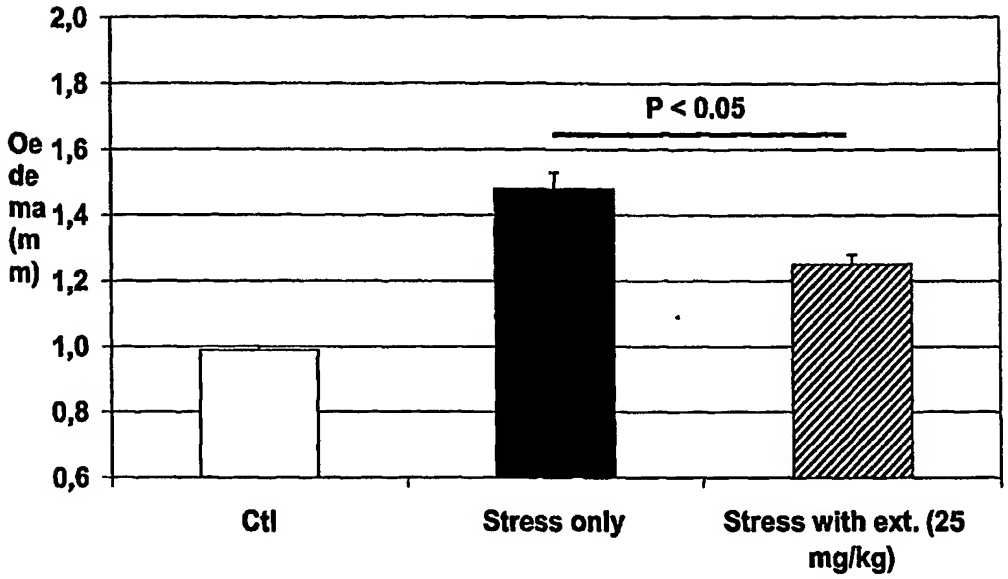

Figure 1

Effect of oral administration (duod num and gavag) on carrag enan-induced paw o d ma

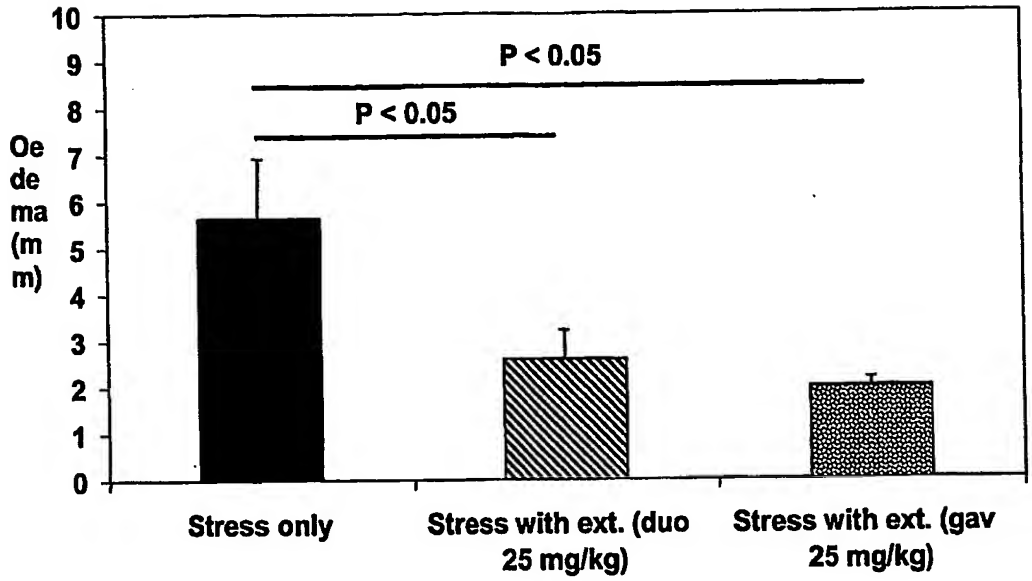

Figure 2